Μικροσκοπία Φθορισμού: Τεχνολογία και Εφαρμογές

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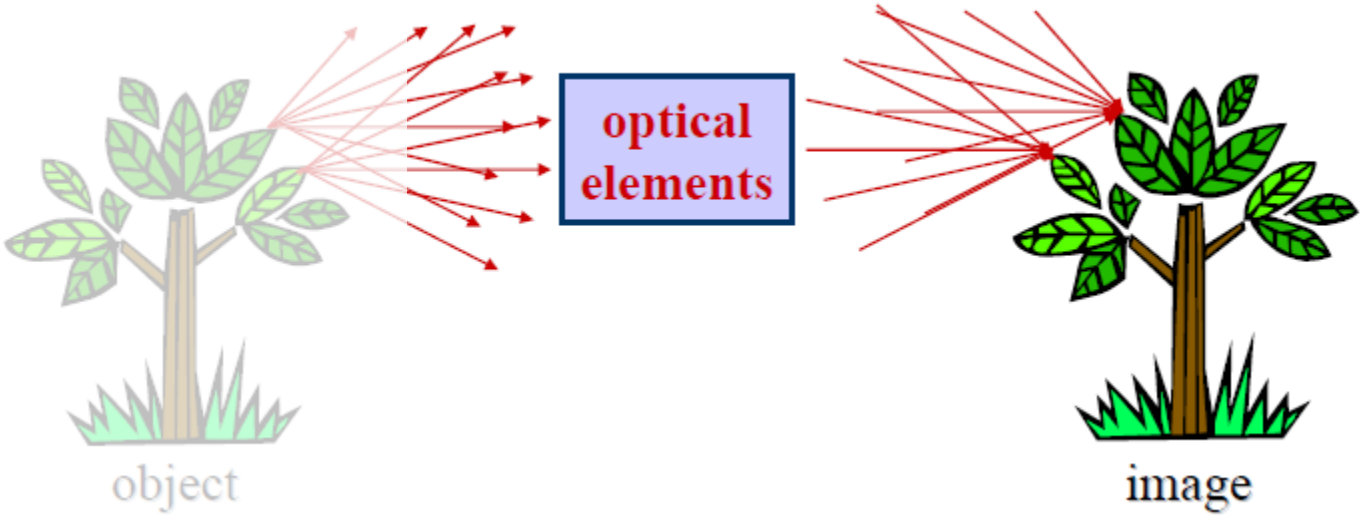
Εμβιομηχανική και Βιοϊατρική Τεχνολογία
Τμήμα Μηχανολόγων Μηχανικών | Ε.Μ.Π.

Χειμερινό Εξάμηνο 2015

The Three Components of Imaging

- Three major components



Emission generation
- Fluorescence

Optical system
- Microscope
- Endoscope

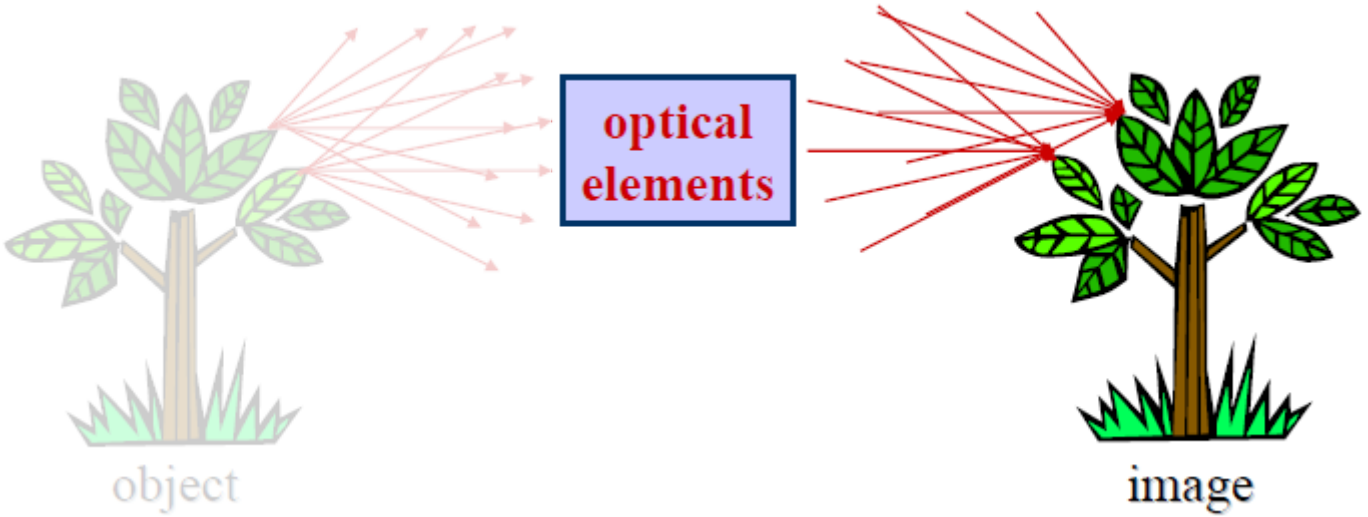
Emission detection
- Detector & sampling

Image by G. Barbastathis, MIT

Presentation Overview

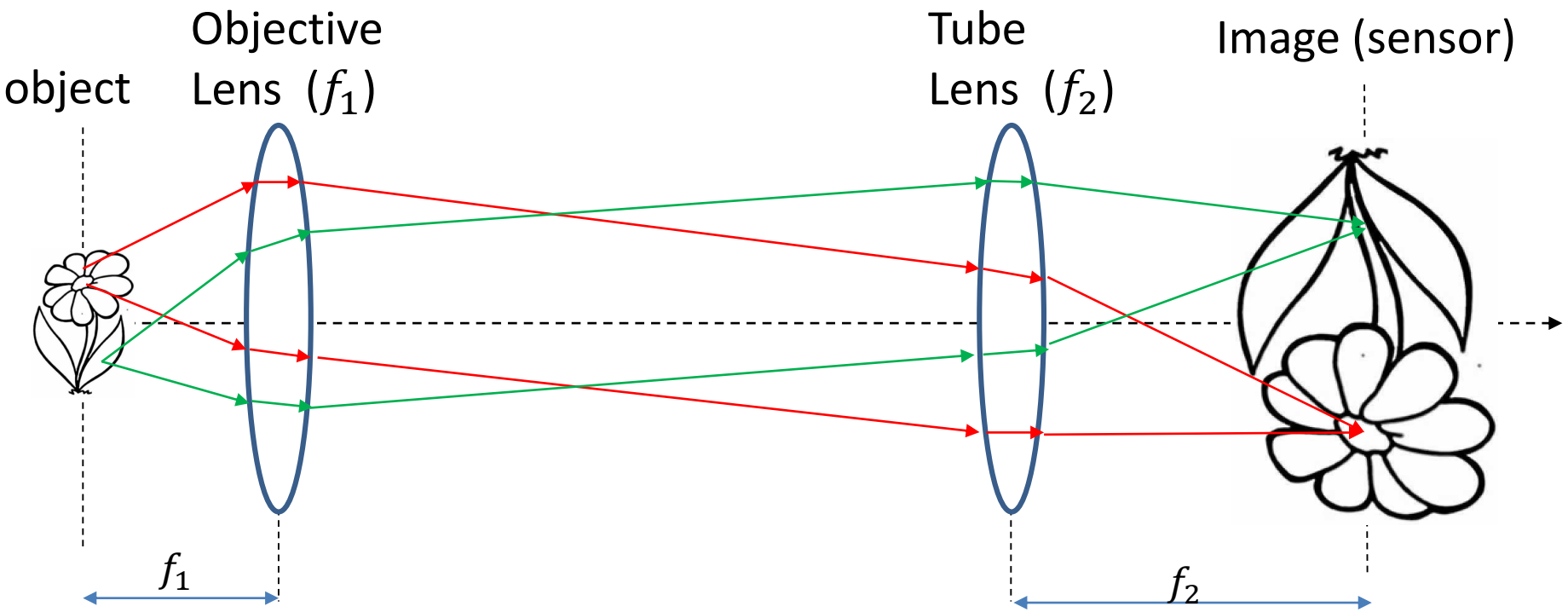
- Microscopy basics
- Standard fluorescence microscopy
- State-of-the-art and Applications of fluorescence microscopy

Microscopy Basics



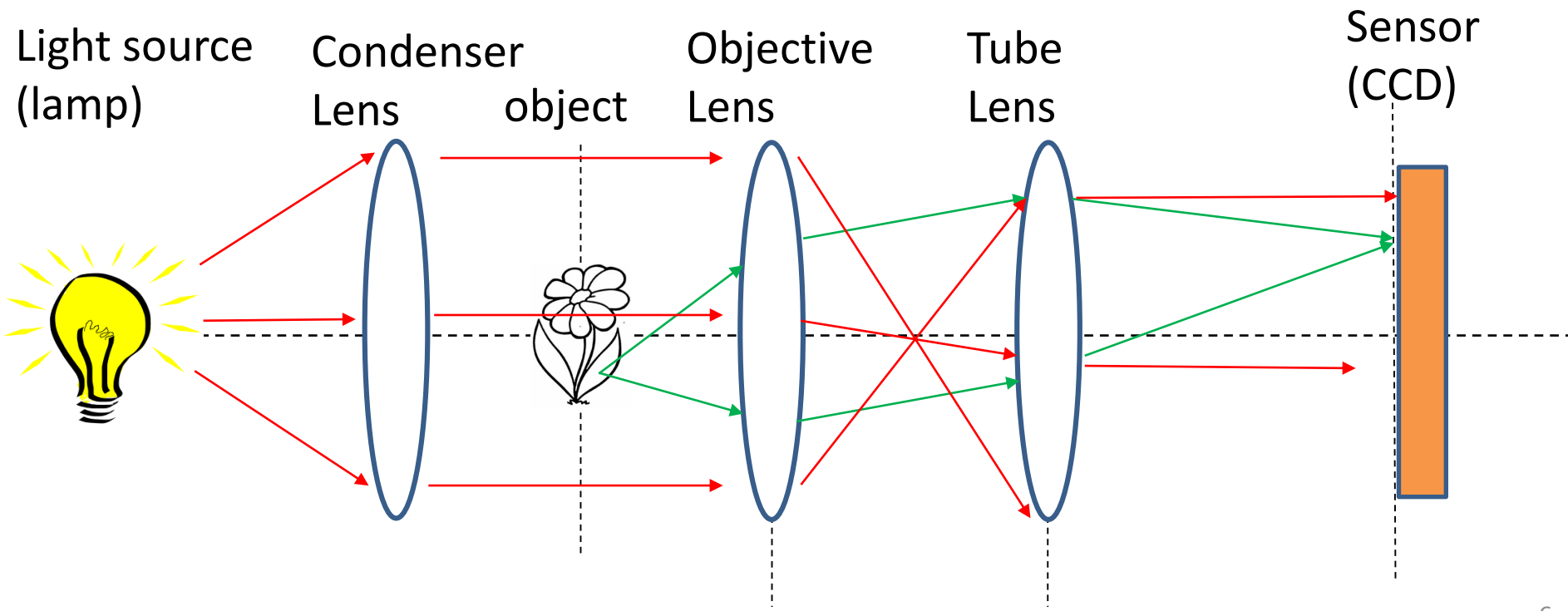
Microscope Principle

- Utilize an optical system to magnify objects
  - Simplest system: 2 lenses
  - Magnification: $M = \frac{f_2}{f_1}$

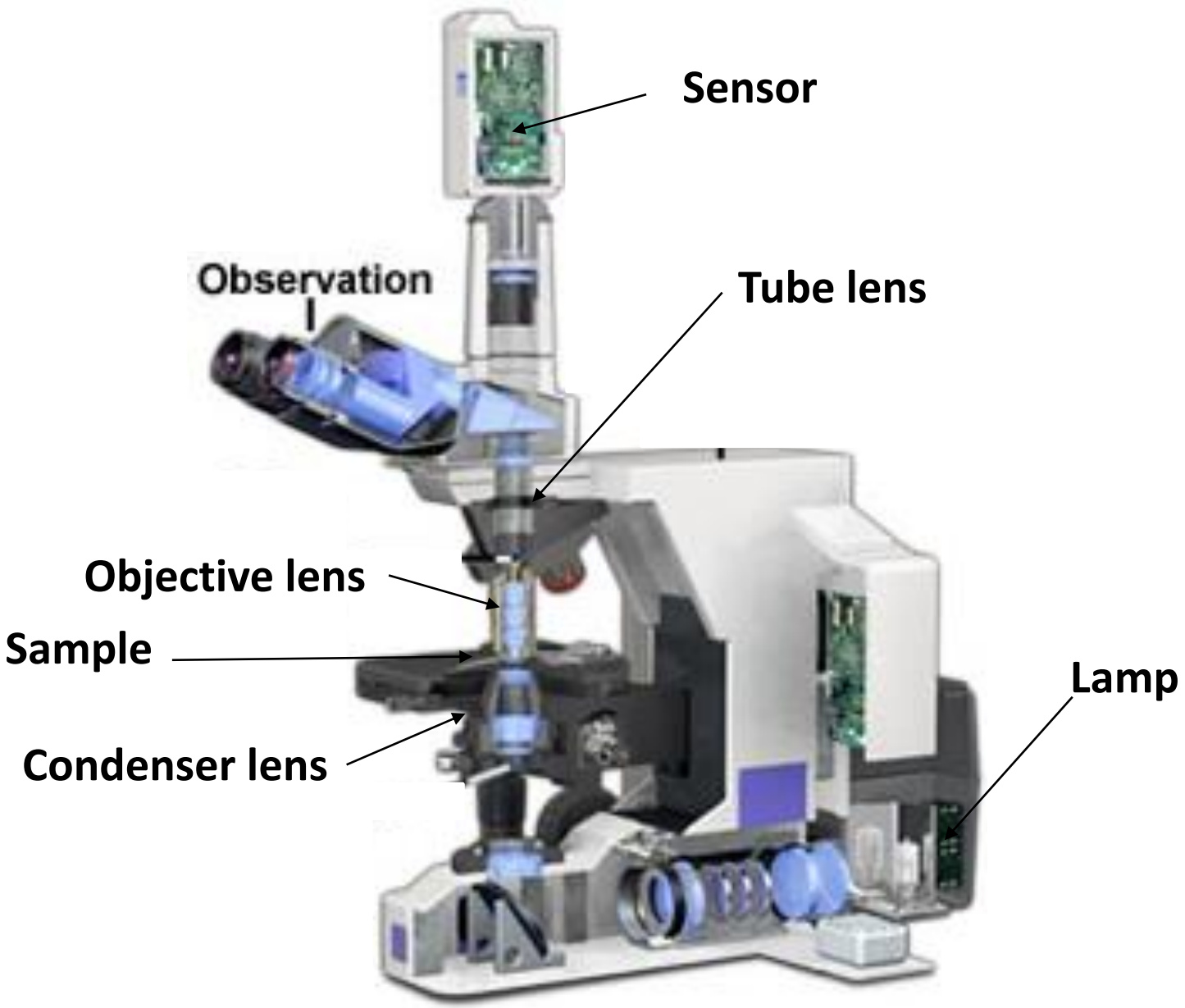


Microscope Principle

- Need a light source (illumination) to generate useful signal (emission) from sample
  - Some illumination is also detected → reduce SNR
  - Ideally would like to detect only emission

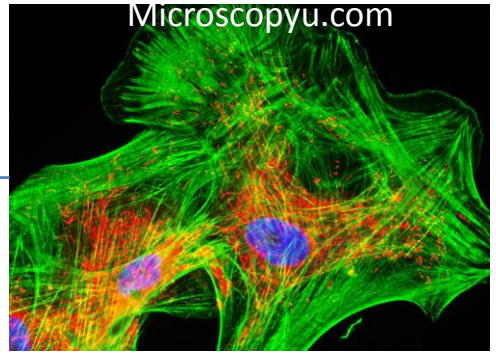


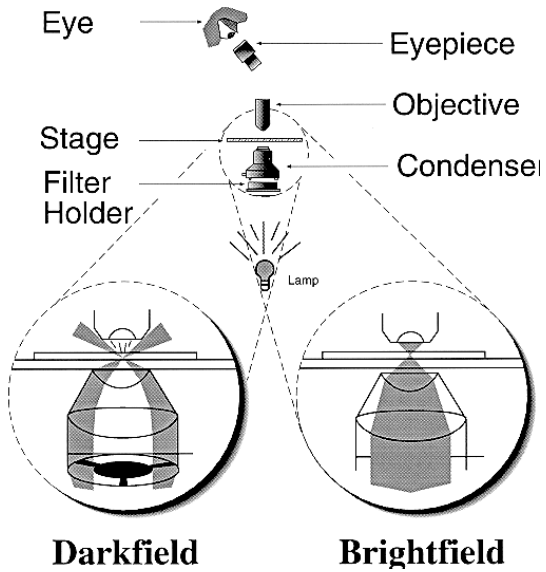
Microscope Uncovered



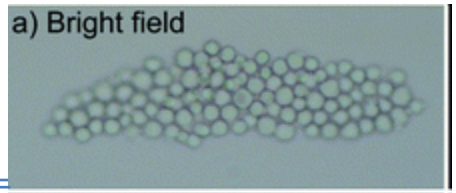
Microscopy Modes Utilized in Biology & Medicine

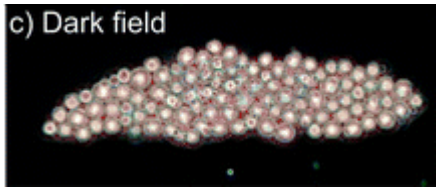
- Modes: ways to generate & detect emission in sample
- Fluorescence microscopy
  - Focus of this presentation
- Brightfield, darkfield microscopy
  - Standard mode
- Phase microscopy
  - Image very thin & transparent samples

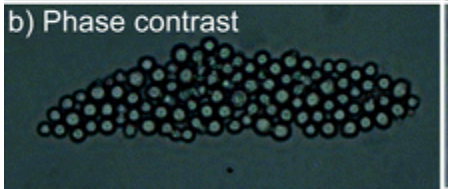




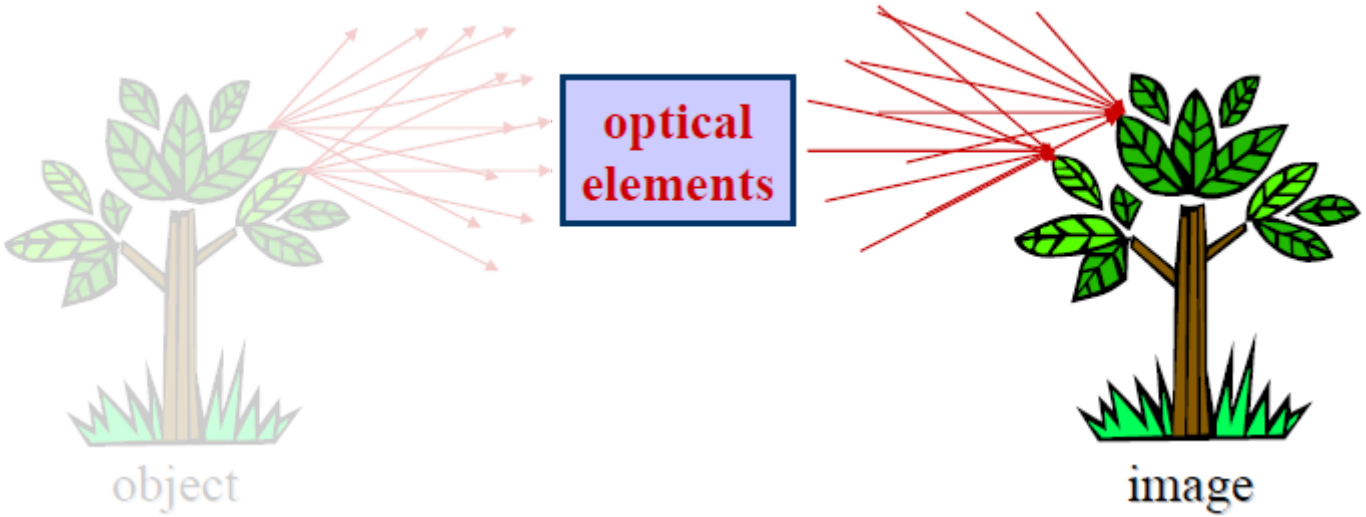
www.wsu.edu







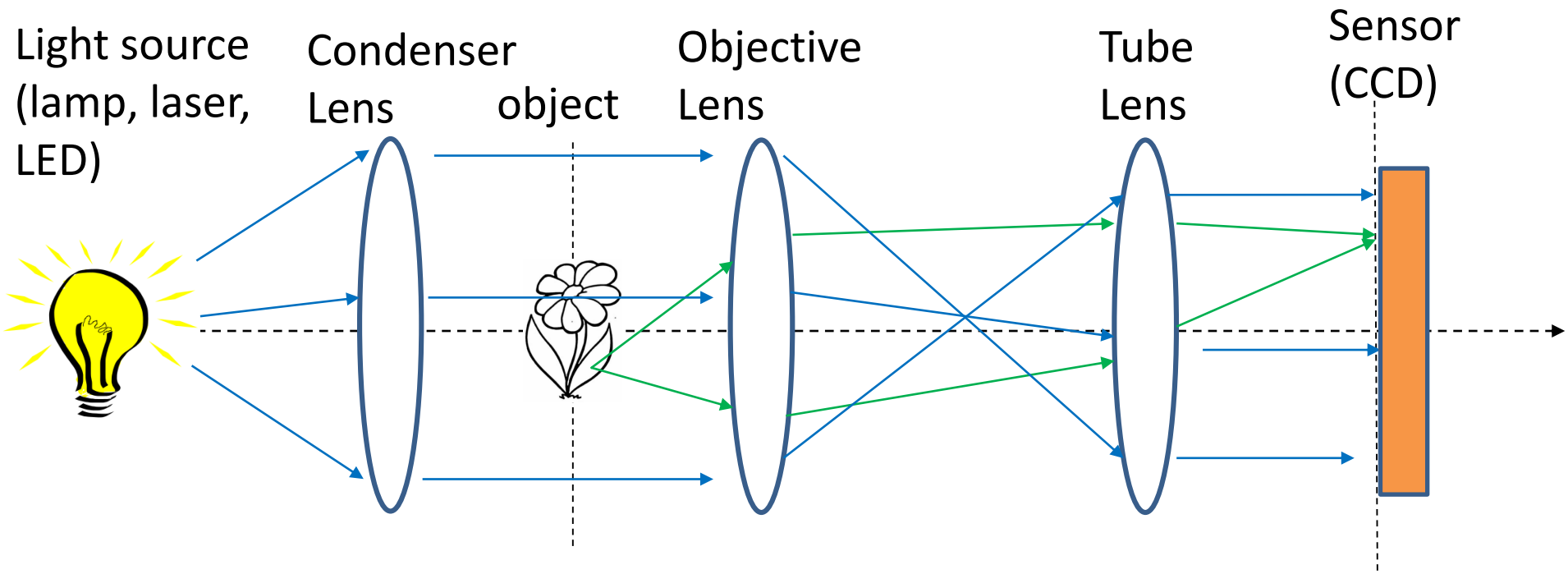
Standard Fluorescence Microscopy



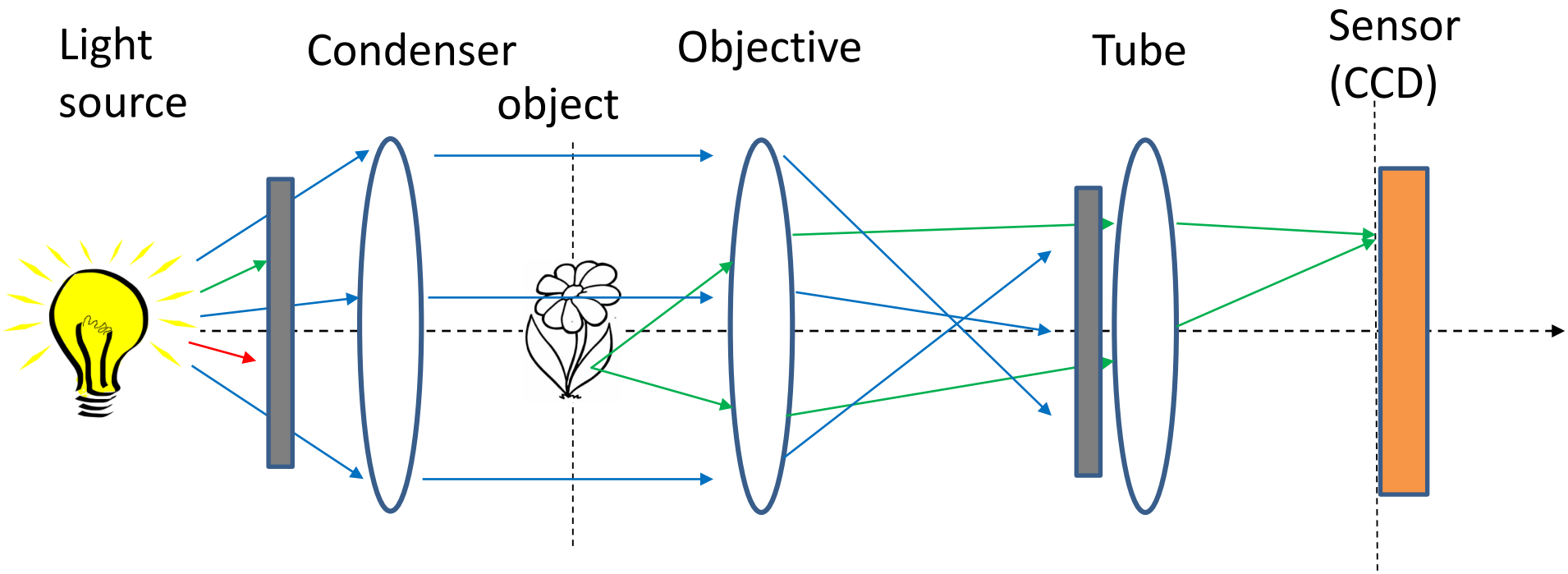
Fluorescence Microscopy

- Standard fluorescent microscope:
  - Easy & popular instrument
  - Relatively cheap (~25-50k)
  - Extremely versatile
  - Can be automated

Fluorescence Microscopy



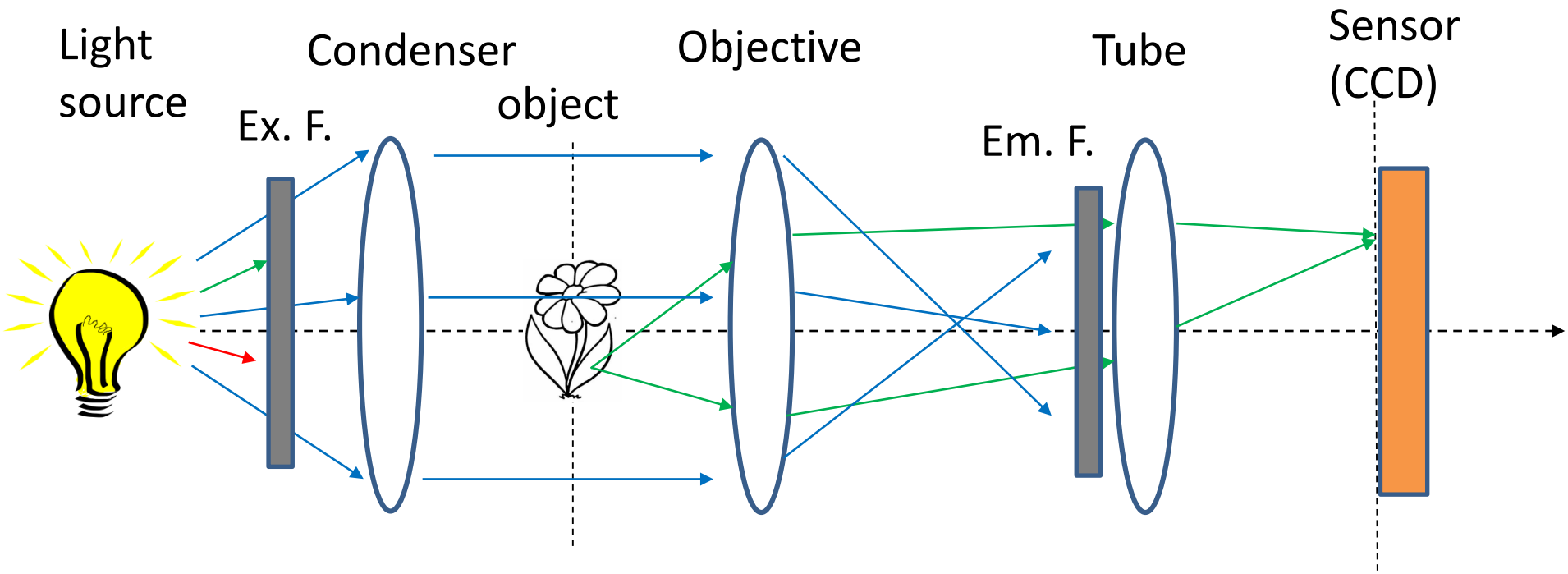
Fluorescence Microscopy



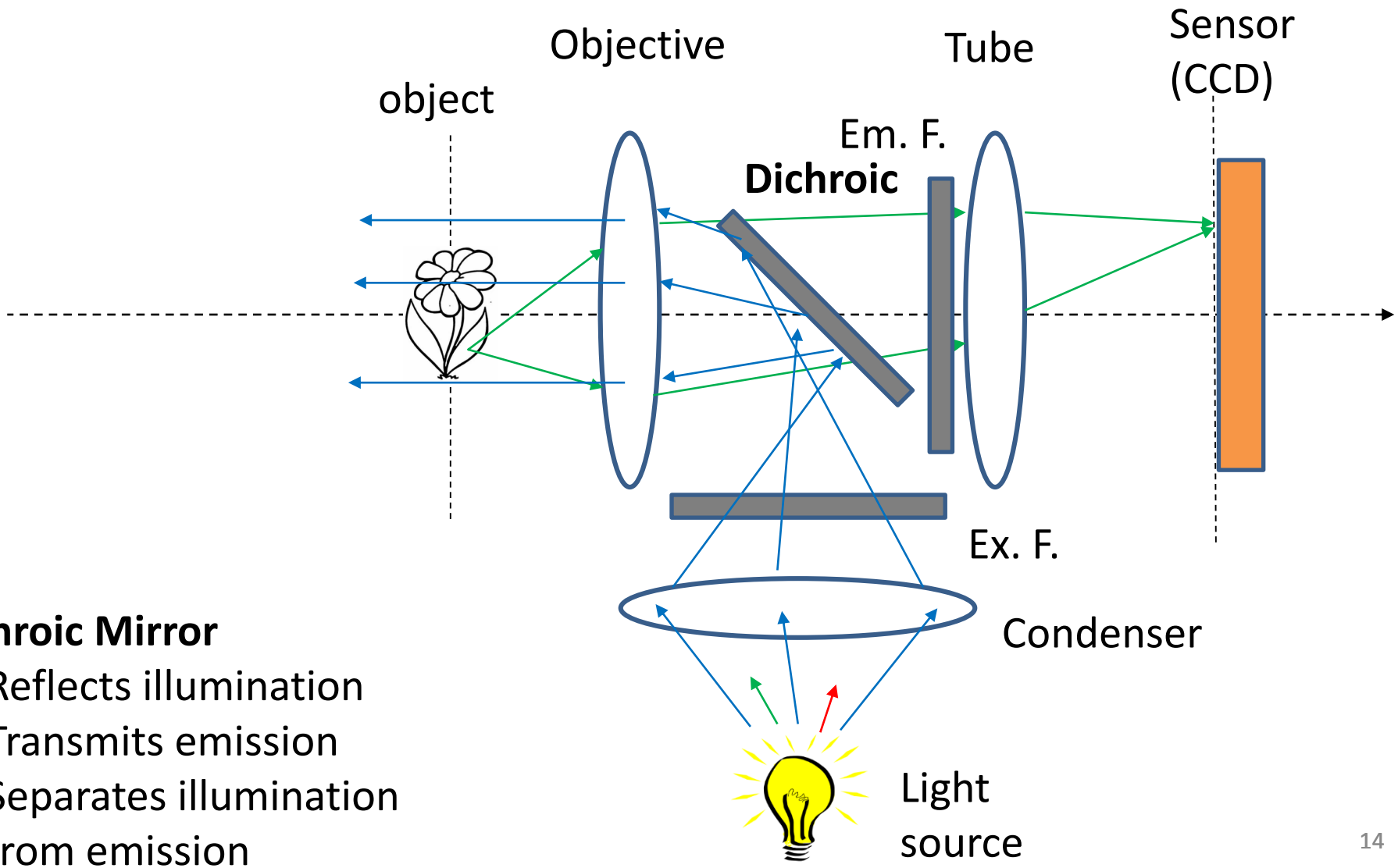
Excitation filter
select wavelength of illumination

Emission filter
Removes illumination light

Fluorescence Microscopy



Fluorescence Microscopy

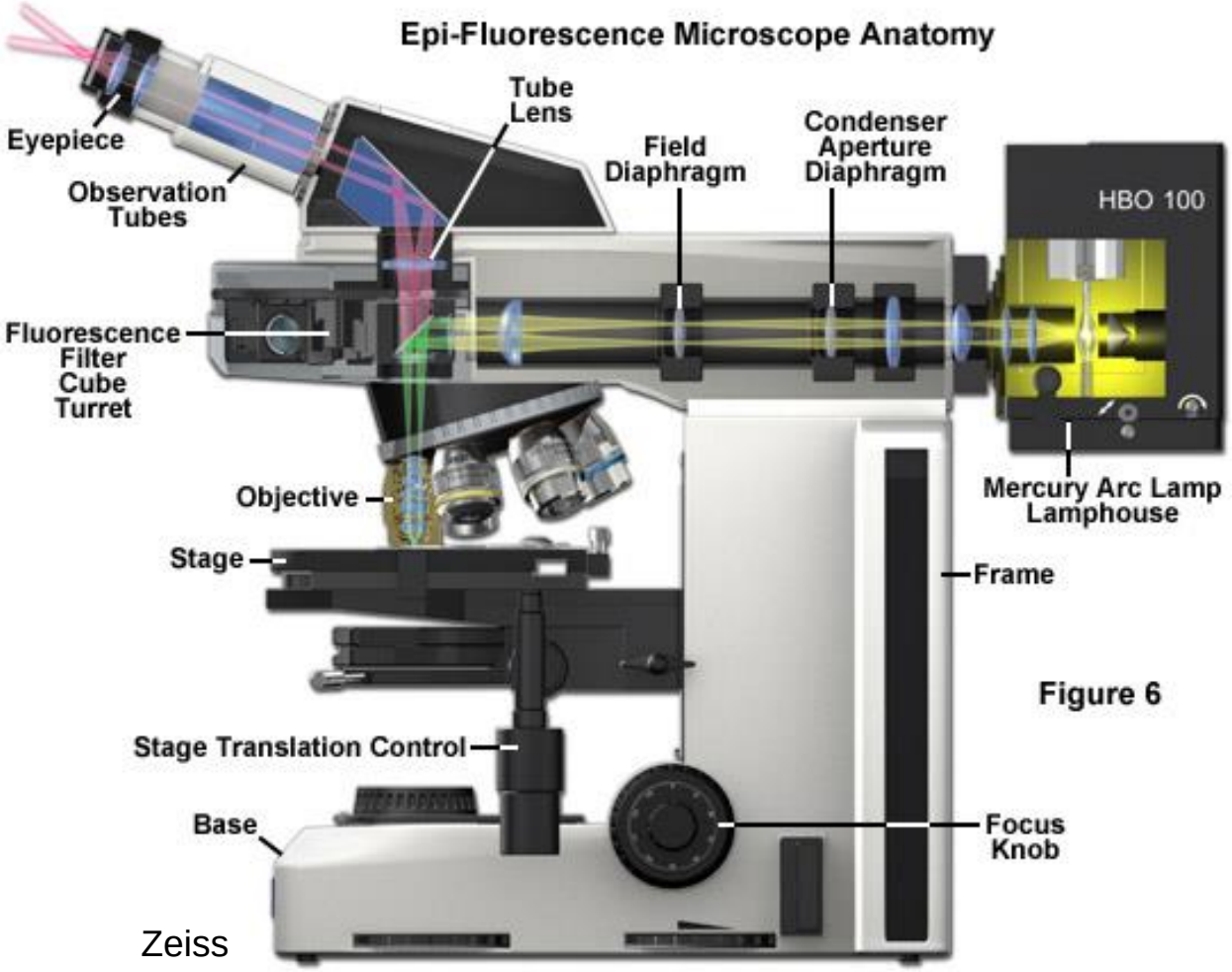


Dichroic Mirror

- Reflects illumination
- Transmits emission
- Separates illumination from emission

Epiflouorescence Microscopy

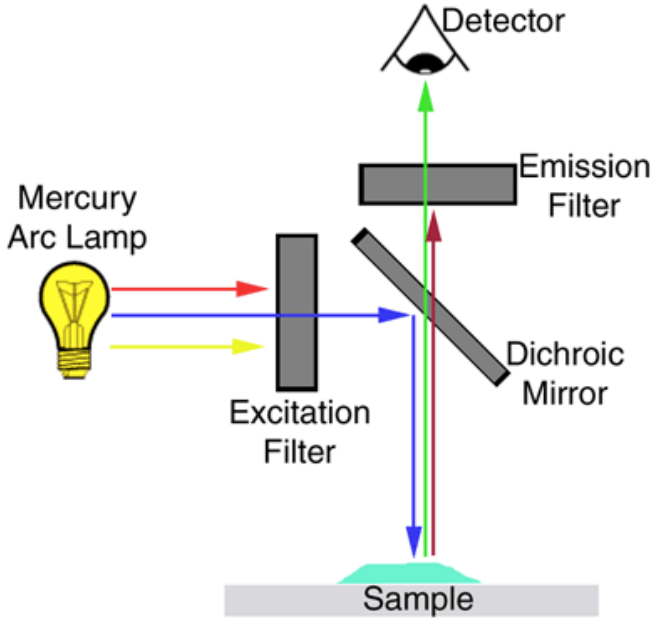
- The objective lens acts also as condenser for the illumination



Zeiss

Fluorescence Microscopy Principle

- A set of 3 optical filters is necessary for fluorescence microscopy
  - Excitation filter: selects wavelength of illumination
  - Dichroic filter: separates excitation from emission
  - Emission filter: removes remaining excitation light in emission path
- 3 filters placed in a “filter cube”
  - Need different filter set for each fluorophore color



http://www.jic.ac.uk

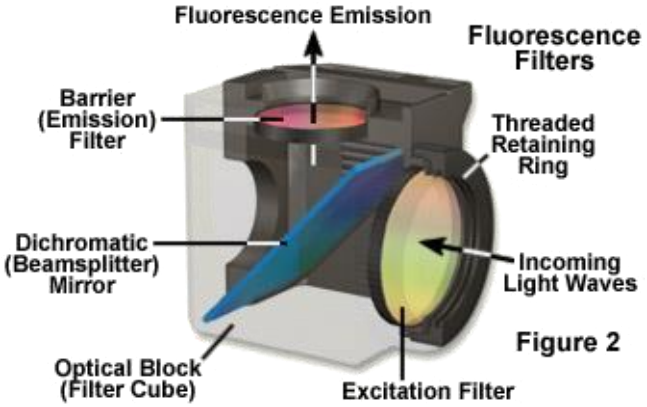
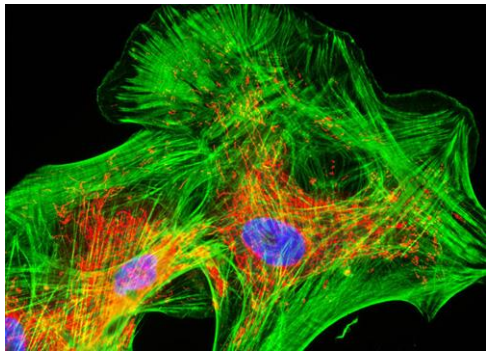


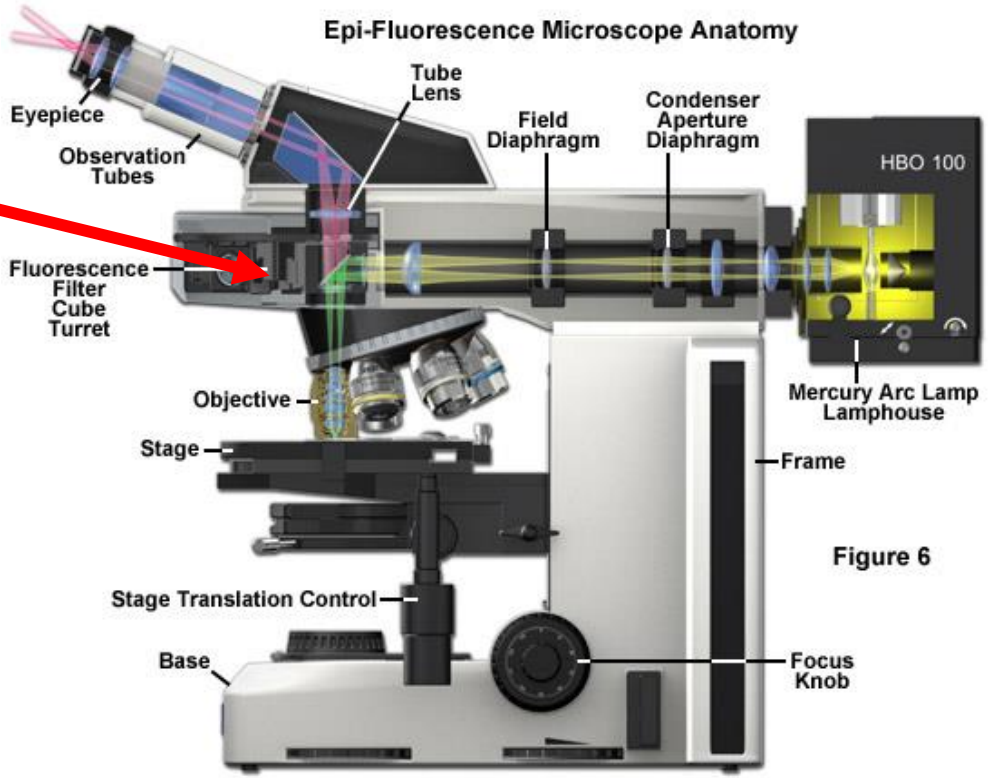
Figure 2

Microscopyu.com

Filter Cubes



- Housed in a filter turrent
  - Pick a different cube for each fluorophore color

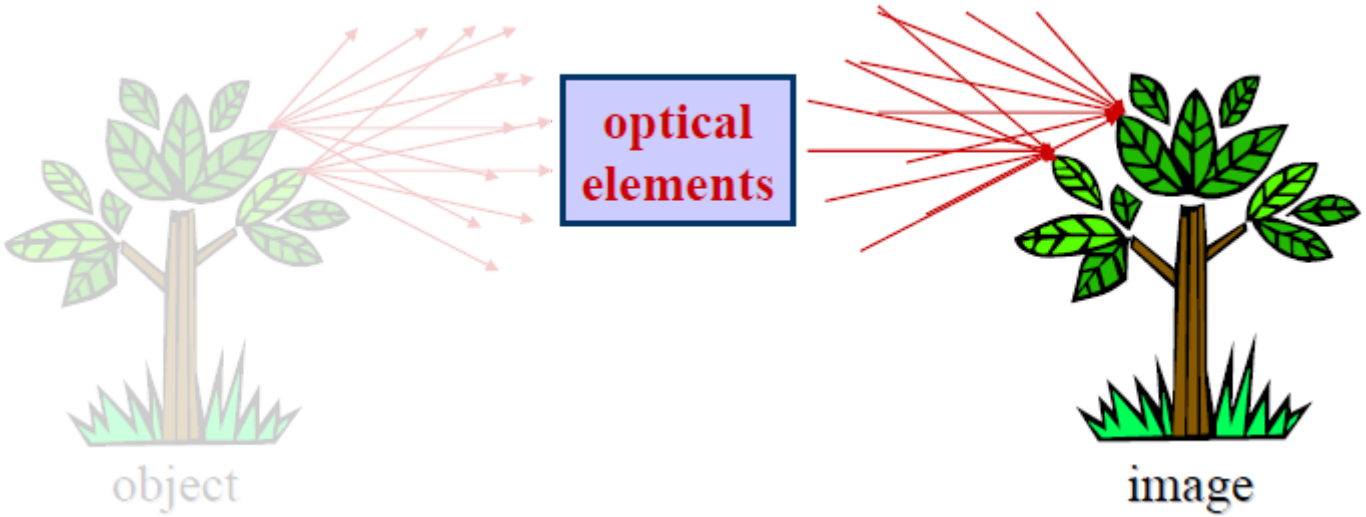


Zeiss

Fluorescence Microscopy Use

- Most modern biology/medical labs possess at least one
  - Ability to visualize expression & localization of any protein of interest
- Multi-color imaging
  - Need multiple “filter cubes”, one for each color

State-of-the-Art in Fluorescence



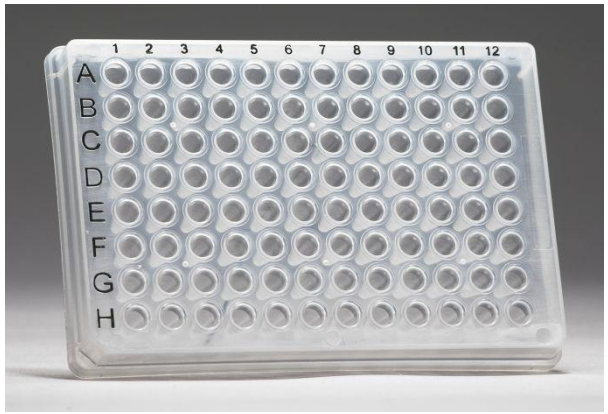
Live Cell Imaging

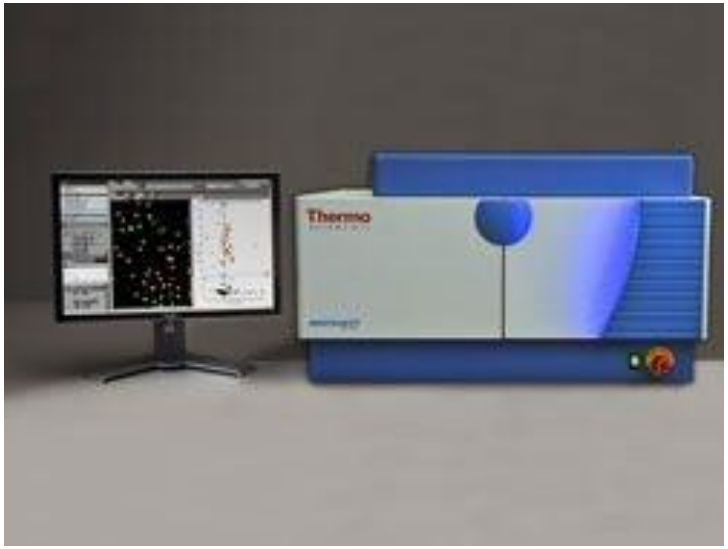
- Enclose microscope in a closet
  - Control temperature, humidity, CO2 content → turn closet into a cell incubator
  - Image live cells as they proliferate/act

Olympus IX81 microscope

Robotic Microscope

- Robotic microscopes that
  - Incubate cells in 96-well plates
  - Image sample over time
  - Able to image thousands of cell samples
  - Expensive (>150k)

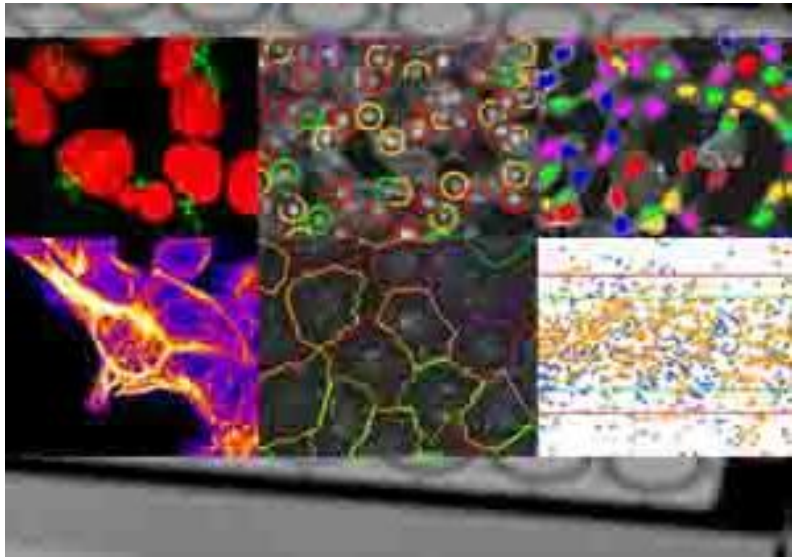




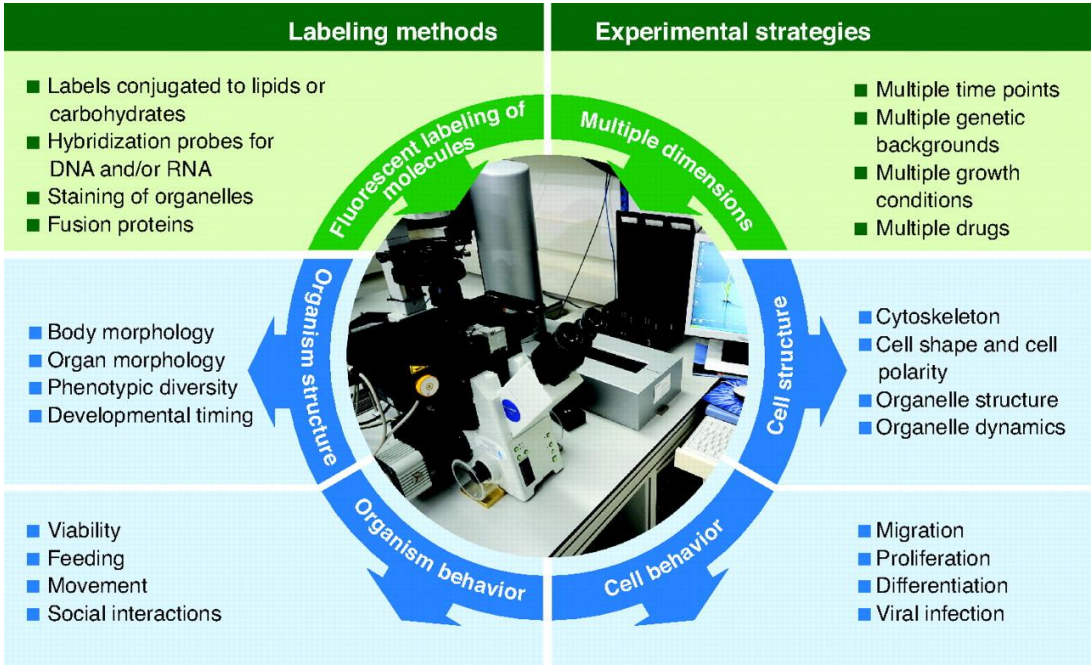
Thermo scientific arrayscan platform

High-Content Screening (HCS)

- Image multiple fluorophores in thousands of samples using robotics microscopes
- Computational tools analyze huge imaging datasets
- Used in drug discovery



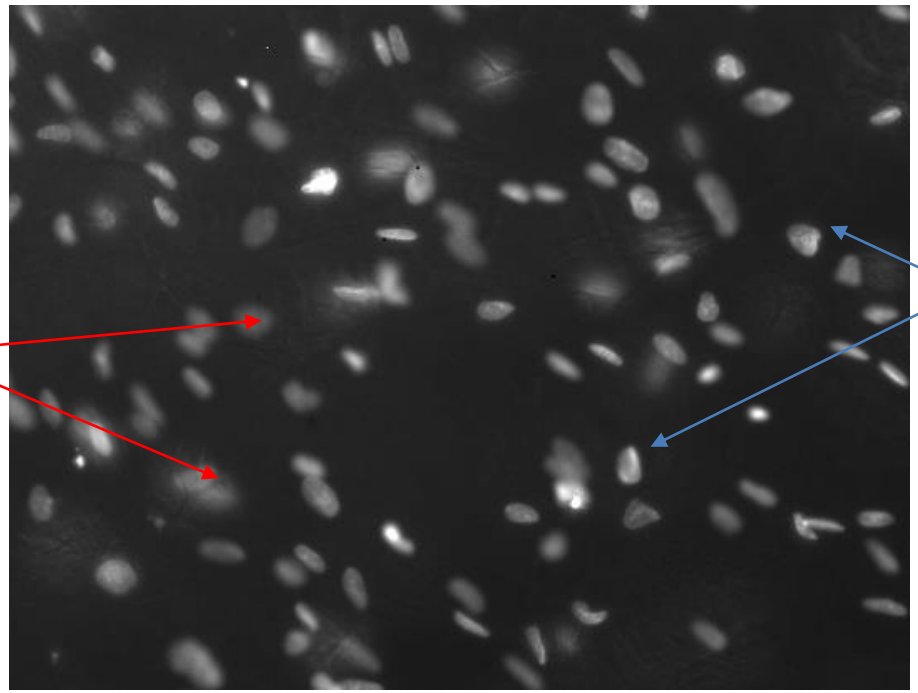
Assay development for HCS (Fraunhoffer.de)



http://jcs.biologists.org/content/124/22/3743

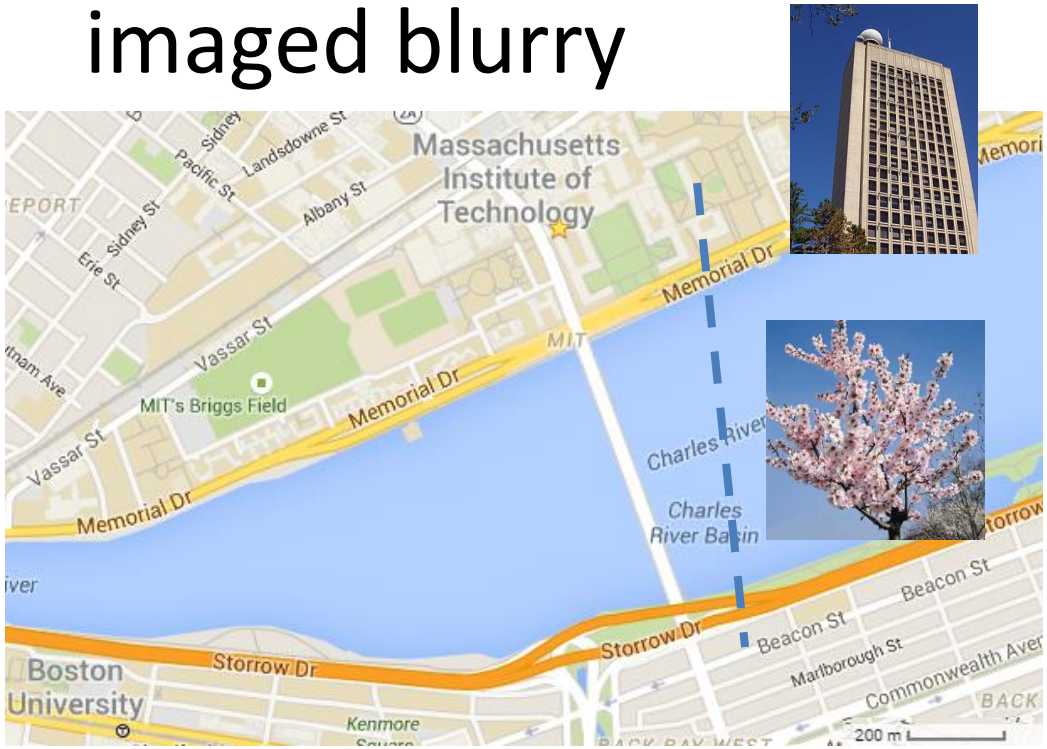
Need to Overcome the Major Limitation of EpiFluorescence Microscopy

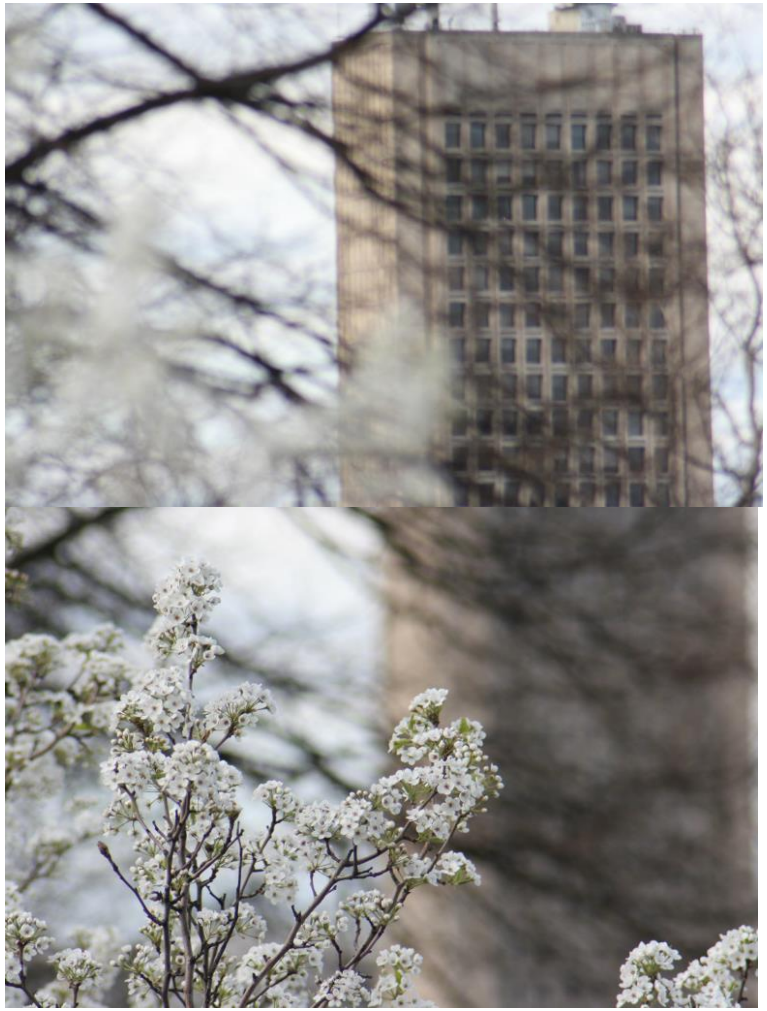
- Epifluorescence microscopy is a 2D imaging method
  - Suffers from out-of-focus light
  - Provides sharp image only of thin specimen (<20 μm)



2D Imaging

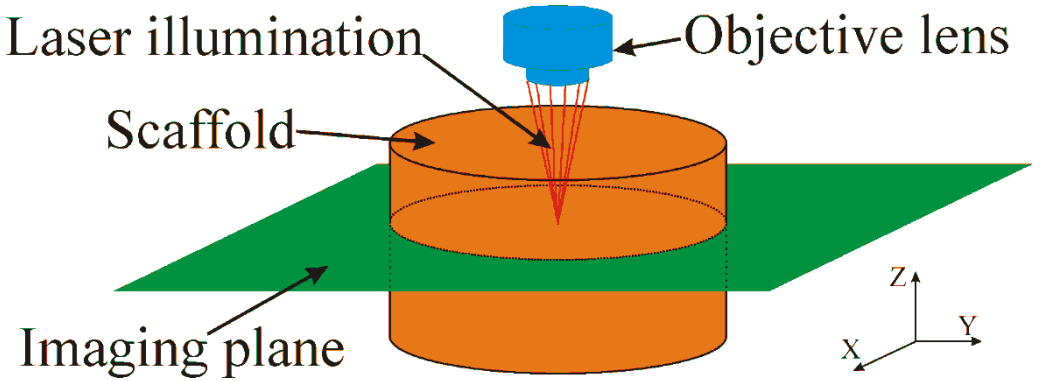
- In-focus objects imaged sharply
- Out-of-focus objects imaged blurry





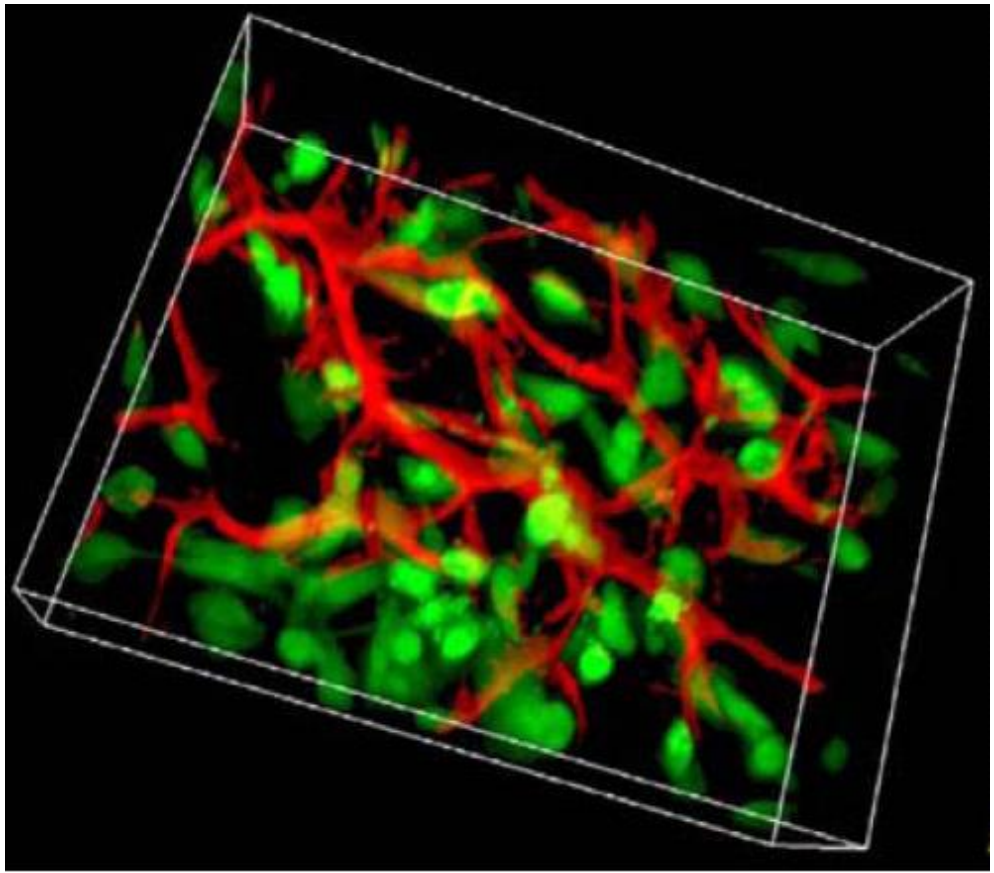
3D Imaging

- In-focus objects imaged sharply
- Out-of-focus objects are NOT imaged
- Image-slicing property
  - Image 1 thin (~1 µm thick) slice of the sample at any exposure



3D Imaging

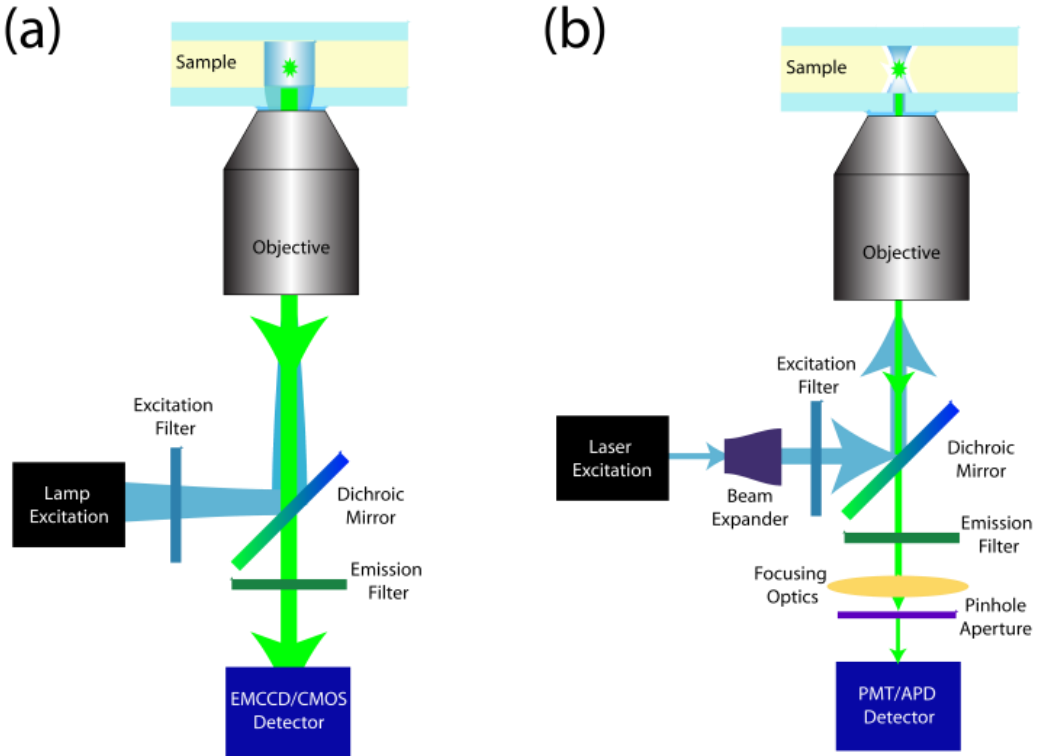
- A 3D image is generated by imaging various planes and combine them into a “image cube”



fibroblasts (green) inside a collagen scaffold (red). Harley, 2006

Confocal Microscopy

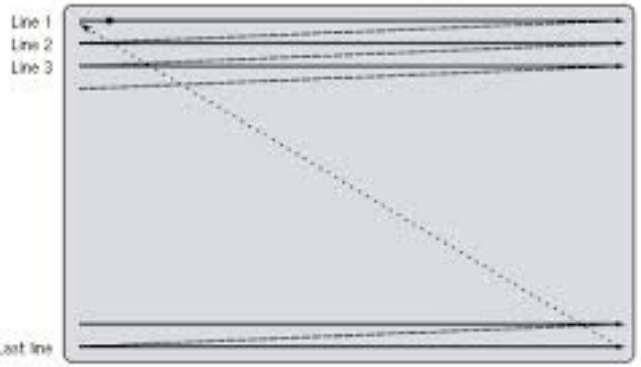
- Optical Slicing is achieved in 2 ways
  - Focus illumination in the sample
  - Place pinhole before detector → reject out-of-focus light
- Use 1 pixel detector
  - PMT, photodiodes



Moran-Mirabal , 2013

Confocal Microscopy

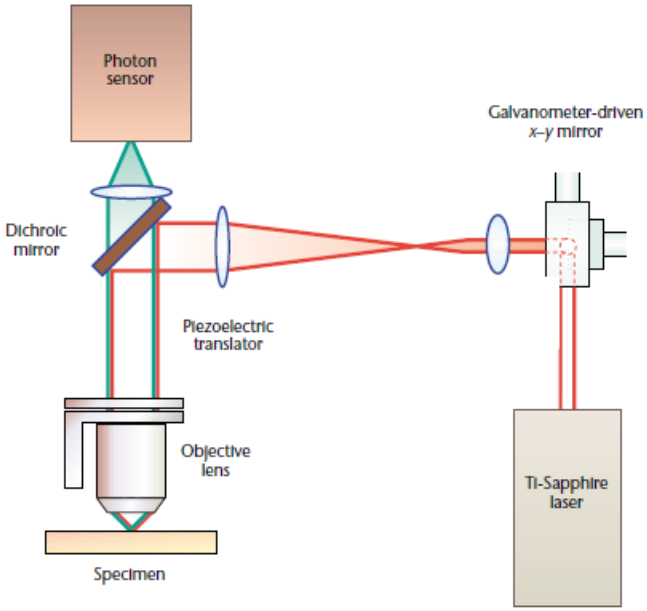
- At each time, a single photon is imaged



- Use scanning mirrors to control (x,y) location of laser focus
  - raster-scanning to image a plane

- Use piezoelectric actuators to shift objective

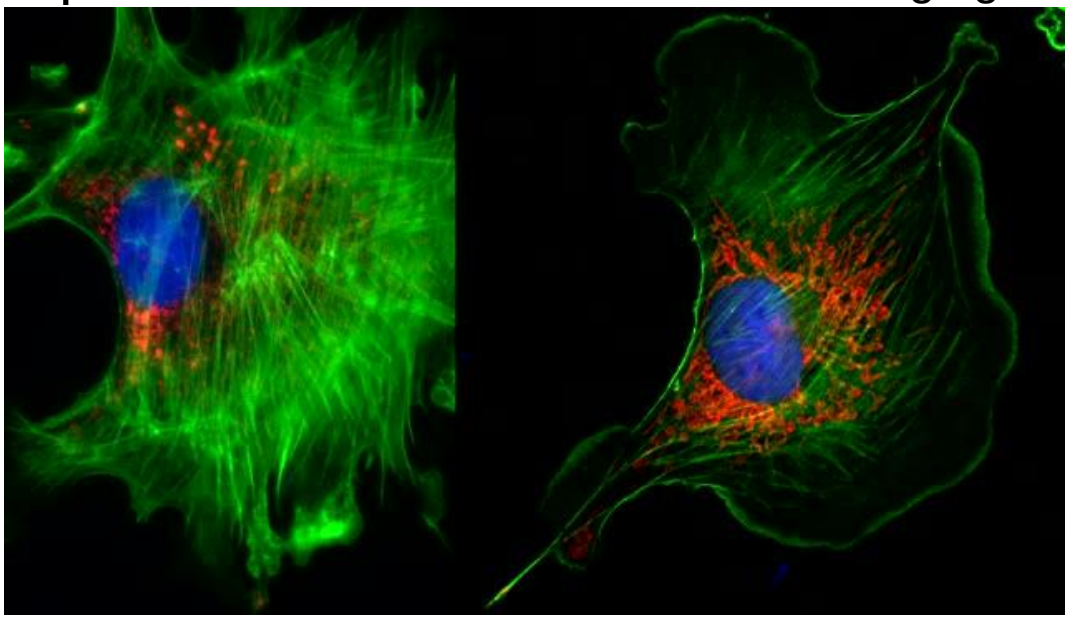
  Sample along Z→ image different planes



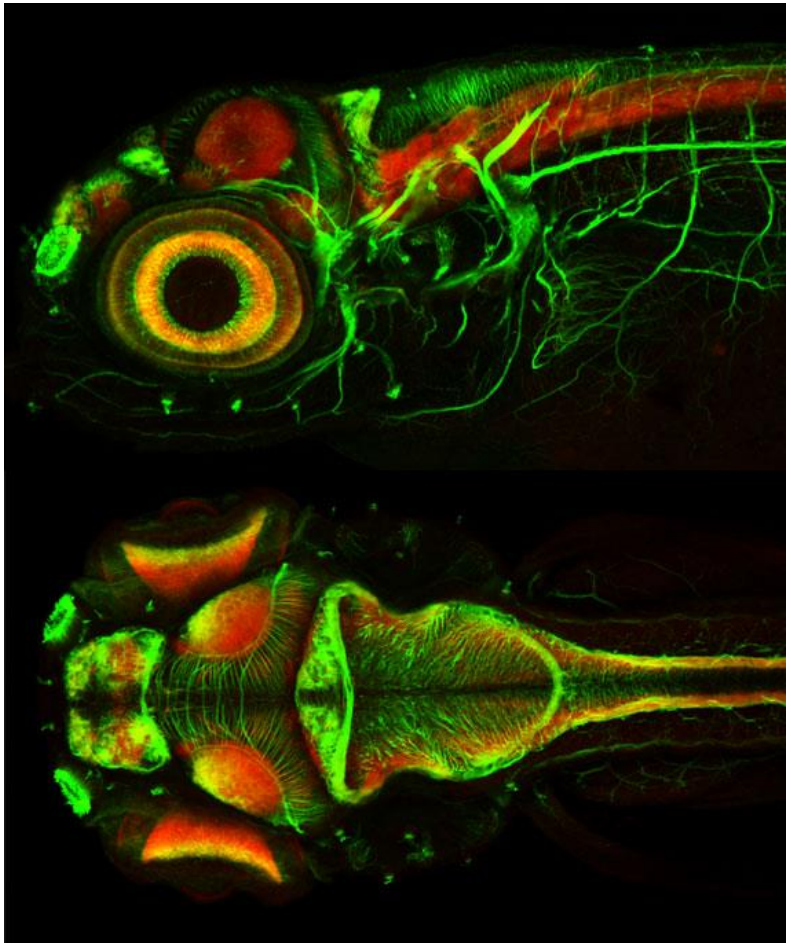
Confocal Microscopy

- Most popular 3D imaging method
- Cost ~30-50k

Epifluorescence Confocal Imaging



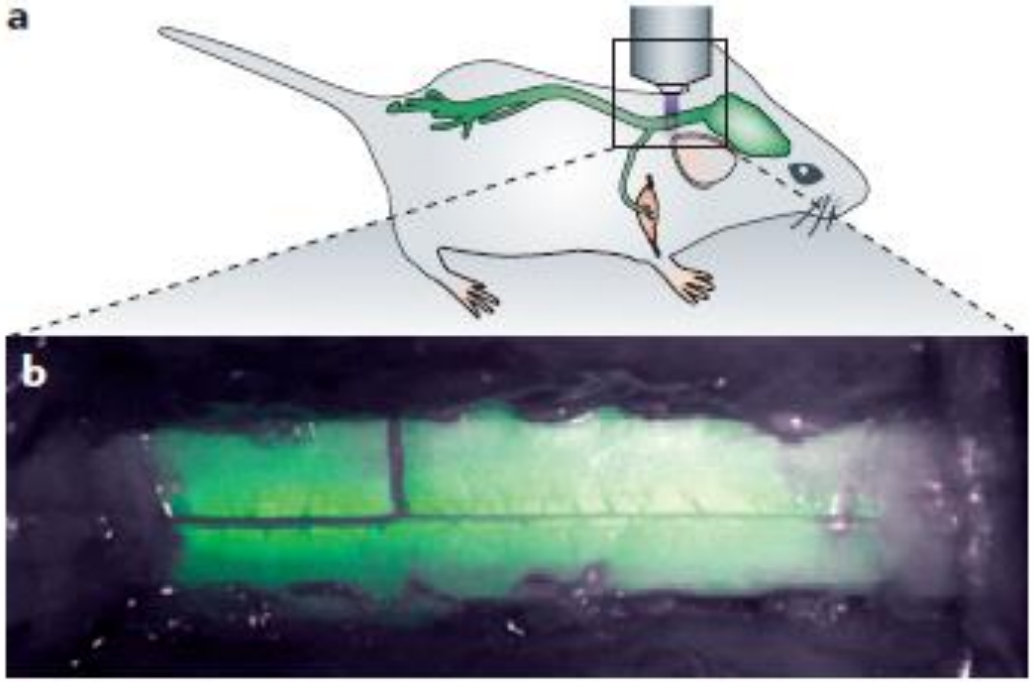
microscopysolutions.ca

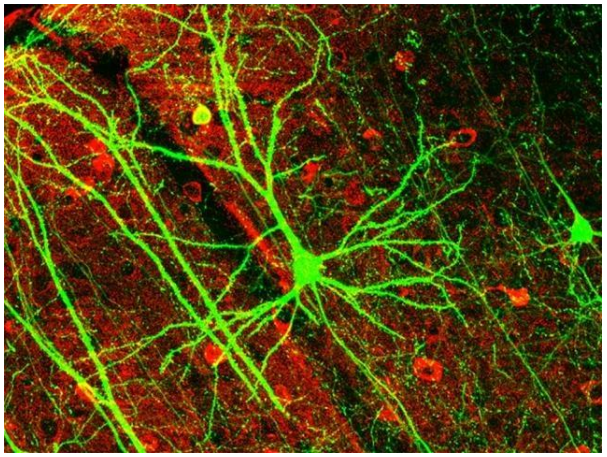


4 day old zebrafish embryo labelled with SV2 and acetylated tubulin antibodies showing axon tracts(green) and neuropil(red). (http://www.ucl.ac.uk/)

In vivo imaging

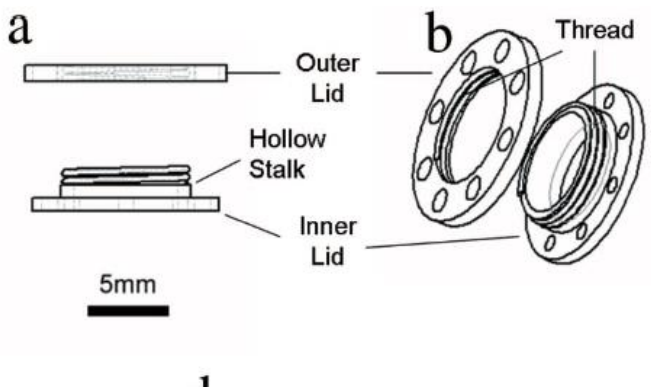
- Advanced 3D imaging methods (nonlinear optics) utilize IR light and provide 3D imaging
  - Enable 3D imaging in live transgenic animals
  - Applications: wound healing, neuron plasticity

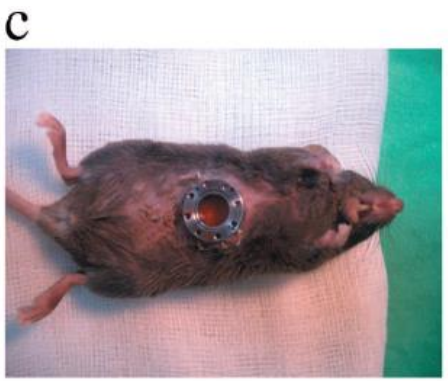


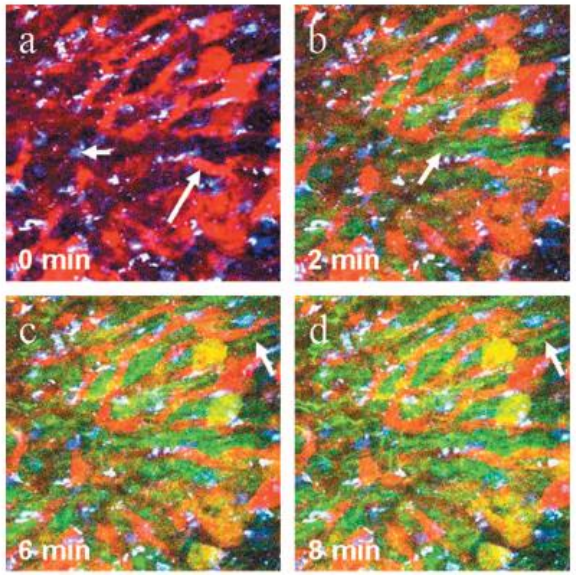


In vivo imaging

- Engineers and doctors collaborate to develop optical devices that enable tissue imaging
  - Imaging cancer biology in animal models

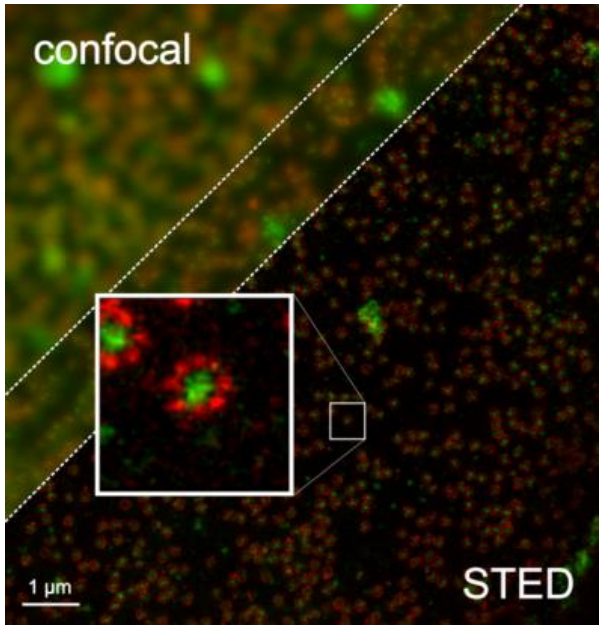


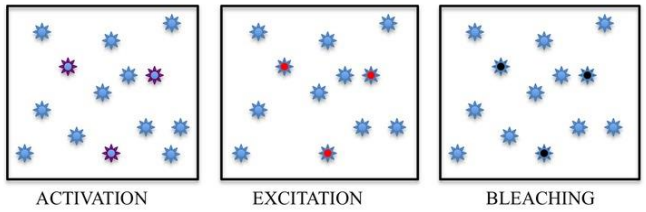




Super-Resolution Microscopy

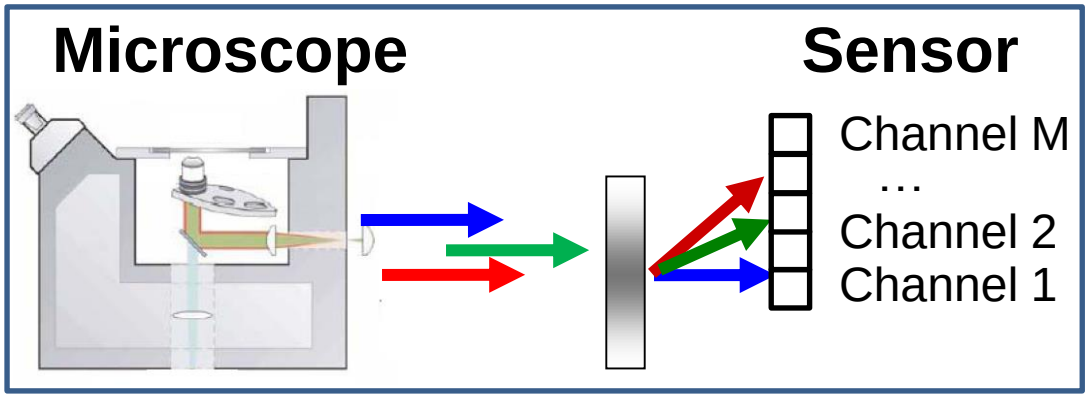
- Methods that provide optical resolution less than $\delta r = 0.61 \frac{\lambda}{\mathrm{NA}}$
- Methods:
  - STED, PALM/STORM
- PALM/STORM microscopy
  - Use photo-activated fluorophotes
  - Light activate a small fraction of fluorophores → image a sub-population of emittors at each exposure → repeat many times



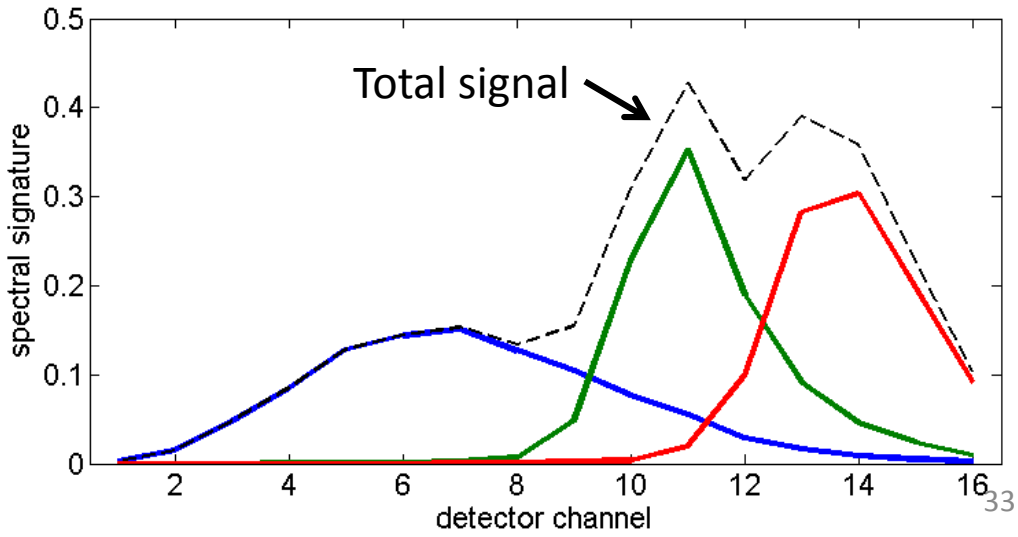


Spectral Microscopy

- Resolve emission of multiple fluorophores based on their emission spectrum
- Use multi-channel detection systems
  - each exposure measures signal at several EM bands
- Use computation
  - Spectral unmixing

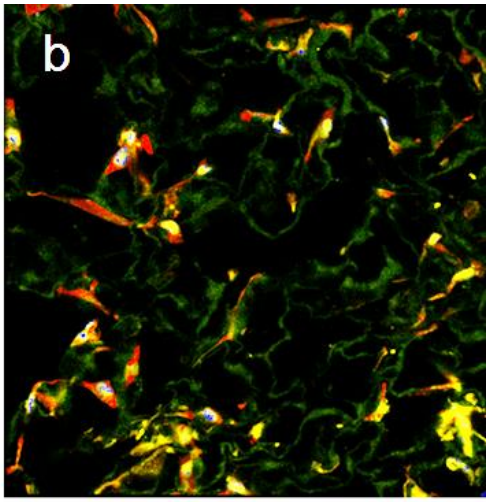


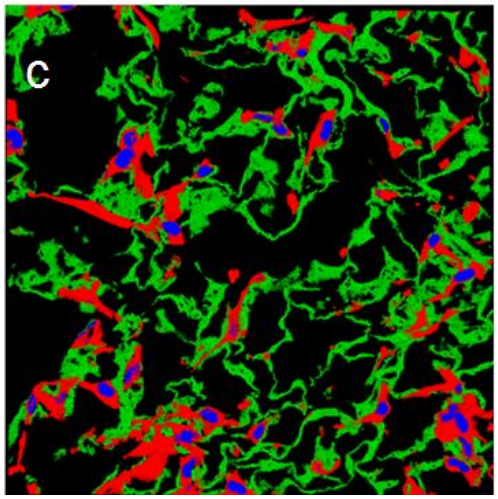
Buelher et al. 2005

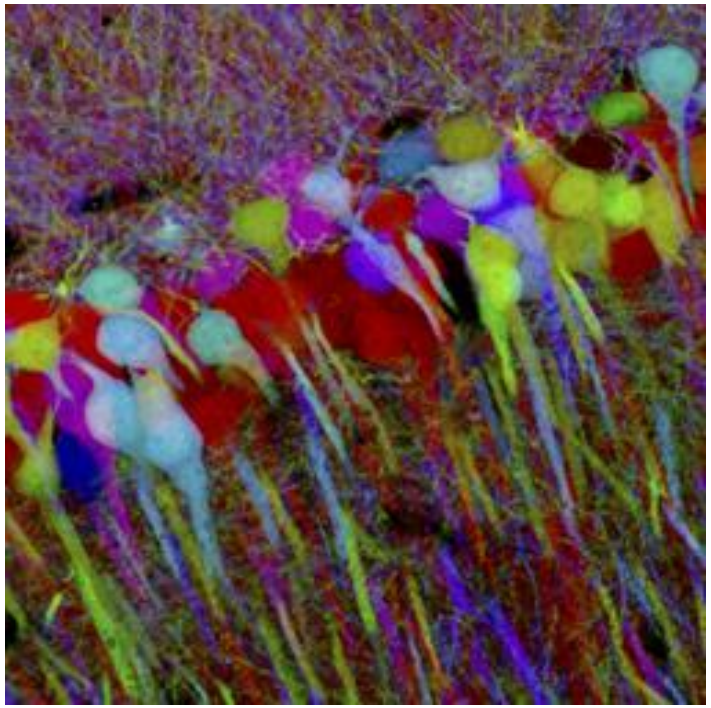


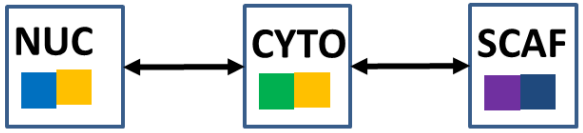
Spectral Microscopy

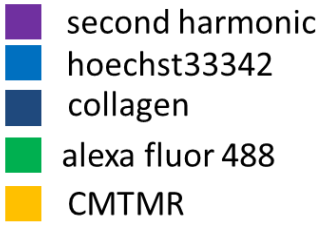
- Based on a single multi-channel image, quantify multiple emittors
  - Accelerate imaging
  - High content







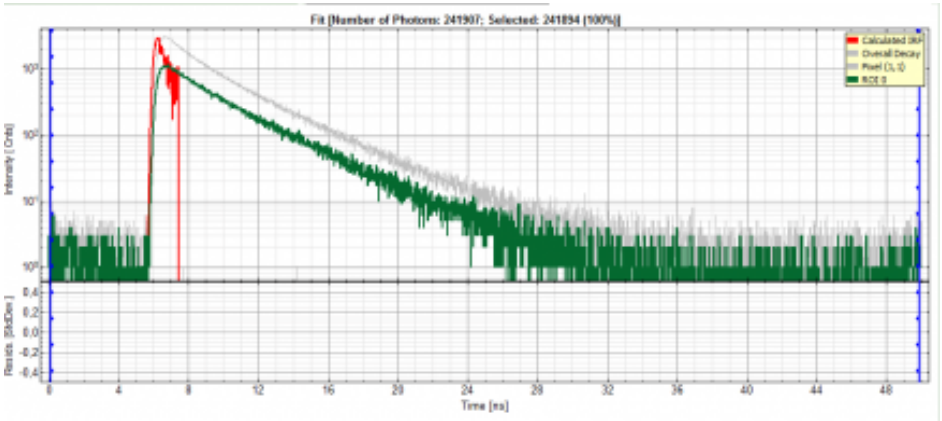




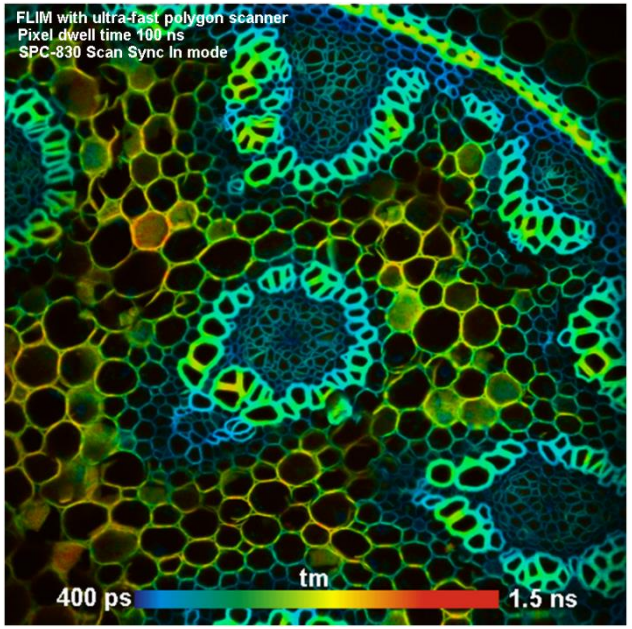
Fluorescence Lifetime Microscopy

- Instruments that can quantify the fluorescence lifetime at each pixel

- Exploit known fluorescence lifetime of each emitter to resolve the emission of each fluorophore



www.tcspc.com



www.becker-hickl.de

ΖΩΗ ΕΠΙΣΤΗΜΗ

Έκαναν το γλυκό πικρό μέσω της χειραγώγησης του εγκεφάλου

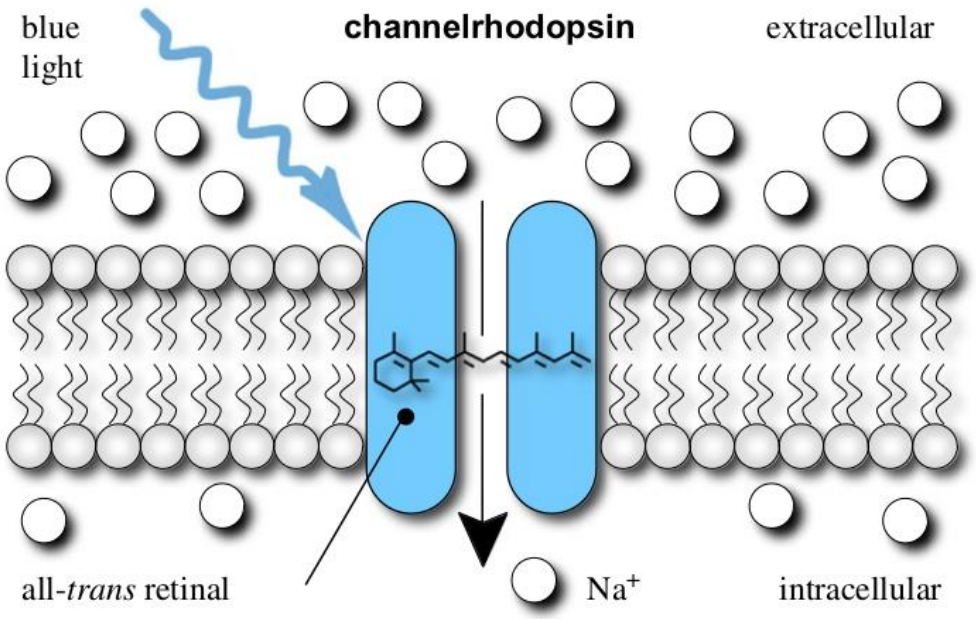
Επιστήμονες παρενέβησαν στον εγκέφαλο πειραματόζωων και με την τεχνική της οπτογενετικής ενεργοποίησαν και απενεργοποίησαν συγκεκριμένους νευρώνες μέσω φωτός λέιζερ και άλλαξαν το είδος της γεύσης τους.

Δημοσίευση: **19/11/2015 - 15:55** Τελευταία ενημέρωση: **19/11/2015 - 15:55** 1 σχόλιο

Αναδημοσίευση από skai.gr

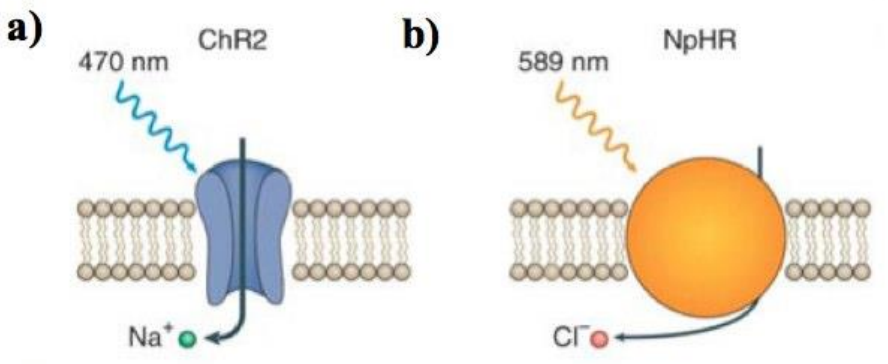
Optogenetics

- Utilize light to activate/de-activate proteins
- Inspired by channelrhodopsin (chR)
  - Protein involved in imaging
  - Absorbs light→ activated→ opens a Na^+ channel

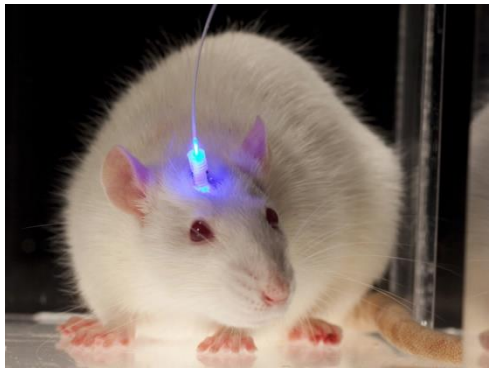


Optogenetics

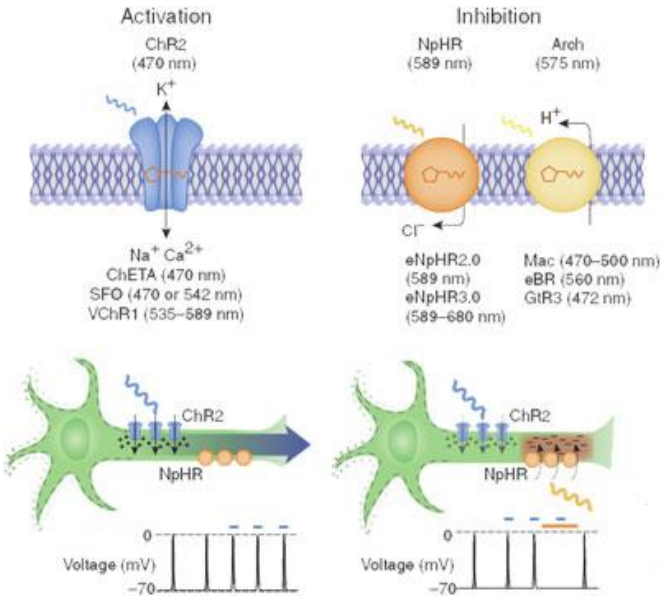
- Biological engineers modified chR → generated novel proteins that upon light activation can do other things
- Then transfect cells or generate transgenic animal that express these proteins



Optogenetics



- Control/probe neuron circuits in brain→study brain
- Use microscope-like instruments to control light



Neuron activatyion/inhibition (nature)